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SOME EFFECTS OF THE WAXY AND SUGARY GENES ON ENDOSPERM DEVELOPMENT IN MAIZE[1]

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INTRODUCTION

Two genetic factors in maize, waxy (*wx*) and sugary (*su*) and their respective dominant alleles, nonwaxy (*Wx*) and nonsugary or starchy (*Su*), so named because of their influence on storage products of the endosperm, are considered in this investigation. Attention is given to the effect of these genes on moisture content, carbohydrate content, weight, and morphology of the kernel; pericarp tenderness as measured by resistance to puncture; and canning quality.

Salisbury (*21*),[3] Atwater (*2*) and Richardson (*20*), working during the last century, demonstrated that mature kernels of sweet corn have approximately the following chemical composition: Carbohydrate, 65–75 percent; fat, 6–9 percent; protein, 10–12 percent. Appleman and Eaton (*1*) found a progressive decrease in total sugars, an increase in fat, and a very great increase in starch during development of the seed. Culpepper and Magoon (*7* and *8*), working with varieties of flint, floury, waxy, dent, and sweet corn, took samples of the growing ears at 5-day intervals from the 5th to the 30th day after pollination for chemical analyses. They stress that a distinctive feature of sweet corn is a high percentage of water-soluble polysaccharides consisting of dextrin and soluble starch. For instance, dent corn had 9.03 percent water-soluble polysaccharides on a dry-weight basis, 20 days after pollination, whereas sweet corn had 28.17 percent. The only other class of corn having a water-soluble polysaccharide content comparable to that of sweet corn was waxy, with 23.65 percent on the 20th day after pollination. However, this proportion fell sharply to 8.30 percent on the 25th day after pollination and remained low, as in dent and floury varieties. Similarly, Brink and Abegg (5), working on mature corn, found no differences in the percentage of water-soluble polysaccharides between waxy and nonwaxy varieties.

Waxy endosperm has been known in corn since 1909 when Collins (*6*) described the character in a new variety from China. Waxy seeds are opaque, light-colored, and if cut show a surface resembling that of wax. Weatherwax (*26*) noted the red staining reaction of waxy-maize starch with iodine and attributed the color reaction to the presence of erythrodextrin. Starchlike substances which give the red reaction with iodine have been found in other species of Gramineae. Meyer

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[3] Italic numbers in parentheses refer to Literature Cited, p. 370.

(*15*) reported that the endosperms of certain rices and sorghums contained some starch granules straining blue, some amylodextrin staining red, and some dextrin staining brown. Harrison (*12*), studying the color reactions between starch and iodine, attributed the red and blue colors to differences in degree of dispersion of the iodine particles. Brink (*4*), using malt amylase as a reagent, reported that waxy and nonwaxy starch are alike with respect to the ultimate sugar unit of which they are composed, namely, maltose. He suggested that the difference between the two starches may be one of degree of association of a common fundamental molecule.

Lampe (*14*) made a microchemical and morphological study of the developing endosperm of maize. She found that presence of the dominant factor (*Su*) in the endosperm cells results in development of rather large, simple grains of carbohydrate, whereas in sweet corn (*su*) compound grains of carbohydrate and globules of liquid dextrin are formed.

Mechanical tests have been used by a number of workers in studies upon maturity of fruits and vegetables, on the assumption that, with increasing maturity, alterations in the tissues occur which affect resistance to puncture. Accepting this idea, Culpepper and Magoon (*7* and *8*) devised a "puncture meter" for corn; this is now produced commercially. Kernels are tested while still on the cob by placing the needle of this instrument at their apex and applying pressure until the pericarp is penetrated Resistance of the kernel to puncture is indicated by the reading just before penetration, when a sharp reaction of the spring and indicator occurs. Culpepper and Magoon found a rapid increase in toughness of corn kernels as development proceeds. They found also that the different classes of corn fall into two distinct groups with respect to toughness: Flour, flint, and waxy varieties become extremely tough as the season advances, whereas the sweet and dent varieties were more tender at corresponding ages.

Doxtator (*9*), studying quality in canning corn by use of the puncture meter, found that inbreds with low puncture pressures gave relatively tender hybrids when crossed with each other. He did not find a significant difference in pericarp thickness among varieties. On the other hand, Bailey and Bailey (*3*) report that varieties with the lowest puncture index at a given stage have the thinnest pericarps. Haddad (*10*) has reported on the morphological development of the pericarp in two inbred lines of sweet corn and their F_1 hybrid. He found that the pericarp decreases in thickness and becomes more resistant to puncture as maturity advances.

EXPERIMENTAL MATERIALS AND METHODS

In order to obtain material for the various comparative studies, waxy sweet corn (*suwx*) was pollinated with 4 types of pollen: Nonsugary, nonwaxy (*SuWx*); sugary, nonwaxy (*suWx*); nonsugary, waxy (*Suwx*); and sufary, waxy (*suwx*). A waxy strain of the hybrid Golden Cross Bantam was used as the seed parent. Golden Cross Bantam is a single hybrid, produced by controlled pollination of two inbred lines of yellow sweet corn, Purdue 39 and Purdue 51, which were developed by Smith (*23*). The parent stocks for the waxy hybrid used in this work were developed by outcrossing to a waxy stock and making the appropriate backcrosses. By 1939 Purdue

39 (*Wxwx*) had been backcrossed 3 times and Purdue 51 (*Wxwx*), 4 times. Some waxy Purdue 51 seed (*wxwx*) was also available. In order to obtain waxy Golden Cross Bantam seed in 1939 for the 1940 studies, heterozygous Purdue 39 plants (*Wxwx*) in the third backcross generation were pollinated with the waxy strain of Purdue 51 (*wxwx*), also 3 generations backcrossed. In the fall of 1939, 41 ears were obtained, segregating 50 percent waxy (*wxwx*) and 50 percent nonwaxy (*Wxwx*) kernels. The waxy kernels, planted in the spring of 1940, gave rise to plants which theoretically possessed 93¾ percent of the germ plasm of the original, nonwaxy Golden Cross Bantam corn. The stock used for studies a year later theoretically possessed 96⅞ percent of the germ plasm of standard Golden Cross Bantam.

As mentioned earlier, plants grown from the waxy Golden Cross Bantam seed (*suwx*) in 1940 and 1941 were pollinated with four types of pollen which differed in the genes for the waxy and sugary characters. Pollen carrying the surgary and waxy genes (*suwx*) was obtained from the waxy selection of Golden Cross Bantam; *suWx* pollen, from a commercial strain of Golden Cross Bantam; *Suwx* pollen, from a waxy double hybrid of the dent type; and *SuWx*, from a strain of field corn. Table 1 shows the genotypes of the pollen applied, of the polar nuclei of waxy Golden Cross Bantam, and of the endosperms obtained for analytical work, as well as the phenotypes of these endosperms.

TABLE 1.—*Genetic character of the material used for endosperm studies*

Pollen applied to waxy Golden Cross Bantam	Polar nuclei of seed parent	Genotype of resulting endosperm	Phenotype of endosperm
SuWx	*suwx*	*SusuuWxwxwx*	Nonsugary, nonwaxy
suWx	*suwx*	*susuuWxwxwx*	Sugar, nonwaxy.
Suwx	*suwx*	*Susuuwxwxwx*	Nonsugary, waxy.
suwx	*suwx*	*susuuwxwxwx*	Sugay, waxy.

Since it was desired to make comparative studies on kernels of the same age, all pollinations were made under controlled conditions on the same day, July 31 in 1940, and July 30, 1941. The number was over 2,000 in both years. In 1940 practically 100 percent fertilization was attained, but in 1941, as a result of excessively high temperature and rain on the day of pollination, the ears obtained were poorly filled.

Ear samples were collected between 4 and 6 p. m. at intervals from the tenth to the seventieth day after pollination. Up to the twentieth day after pollination in 1940, 15 ears for each endosperm type were picked on each harvest date, but after the twentieth day, when the ears had increased considerably in size, the number was reduced to 10 ears per type. In 1941, however, because of a poor seed set, 25 ears for each endosperm type were taken during sampling. Ears for canning tests were collected on a larger scale as a separate experiment in 1940.

The samples were taken to the laboratory and husked. Tests were made on resistance of the pericarp to puncture by use of the puncture meter. Needles of 3 diameters were available for the puncture meter. In 1940 the medium size (diameter =0.048 inch) was used, and in 1941 the small size (diameter =0.025 inch). The kernels were then cut from the cob with a scalpel. Samples were taken for moisture deter-

mination and a few kernels were preserved in formalin-acetic-alcohol for histological study. In 1940, 100 kernels from each of 5 ears for each endorsperm type were taken for moisture samples. Evaporation was carried out in a Proctor-Schwartz over at 80° C. for 48 hours. In 1941, 3 composite moisture samples for each kernel type were taken from 25 ears. The remaining kernels were dried for 24 hours at 80° C. under forced draft and stored in half-pint milk bottles for use in carbohydrate analyses.

Chemical determinations were made of reducing sugars, total sugars, and water-soluble polysaccharides in the 4 kernel types at 12 stages of development in 1940 and at 8 in 1941. The dried kernels were cleaned of chaff by compressed air, broken to small pieces in a drug mill, and ground in a ball mill to pass an 80-mesh screen. Extraction of 5-gm. samples in 80 percent alcohol brings the sugars into solution. To obtain polysaccharides soluble in cold water, the residue from this extraction was transferred to flasks and 200 cc. of distilled water added and thoroughly shaken. The milky liquid was allowed to stand for 12 hours at room temperature and was then filtered by suction through infusorial earth. Inversion of sucrose and water-soluble polysaccharides was accomplished by concentrated HCl (specific gravity =1.1878). All sugar determinations were made by Bertrand's volumetric permanganate method as outlined by Heinze and Murneek (*13*).

The ears remaining after sampling was completed in 1940 were used to ascertain comparative dry weights of the 4-kernel types. Two hundred ears of each type were harvested at the 72-day stage, classified according to the number of kernel rows per ear, and shelled. Approximately 70 percent of the ears had 12 rows of kernels; 15 percent, 10 rows; and 15 percent, 14 rows. For each of these 3 classes of ears within each of the 4 endosperm types, 1,000 kernels were weighted and the weights corrected to a dry basis.

EXPERIMENTAL RESULTS

CARBOHYDRATES

The different types of corn do not vary significantly in the quantities of reducing sugar which they contain or in the rate of change of this carbohydrate during development. Since the results obtained in 1941 closely approximate those obtained in 1940, only figures for the latter year are presented. The sugary kernels generally showed a higher content of reducing sugar than the nonsugary kernels (table 2 and fig. 1). On the other hand the waxy gene did not have a uniform influence on the percentage of reducing sugars present. All four percentage curves dropped sharply up to the 16-day stage in 1940 and then leveled off under 3 percent. In 1941 the reducing sugar had already declined to below 5 percent by the 14-day stage, the first date of sampling. Prevailing high temperatures and clear days between time of pollination and first harvest unodubtedly contributed to this low amount of reducing sugar in 1941.

Total sugars were present in greatest quantity on the first harvest date and decreased continuously thereafter in both 1940 and 1941. The rate of decrease was rapid until the 16-day stage and more gradual thereafter (table 2 and fig. 1). The sweet kernels maintained a

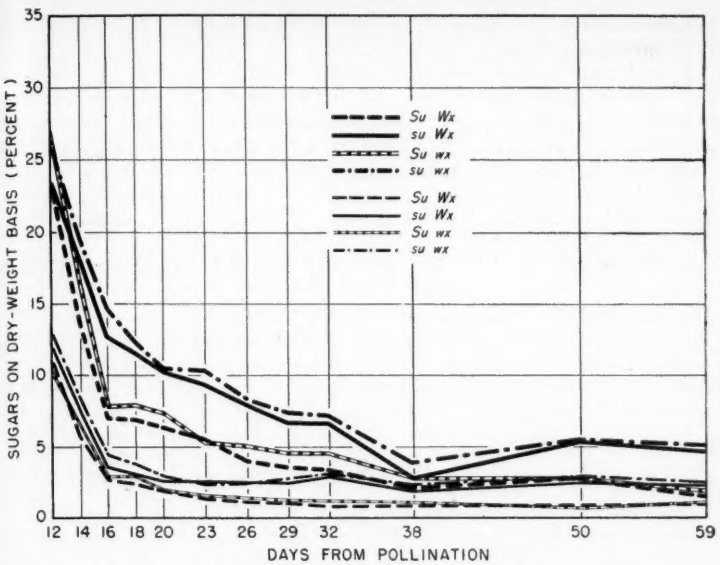

FIGURE 1.—Curves, showing the changes in sugar content for four kernel types during development in 1940 (heavy lines represent total sugars and light lines, reducing sugars).

TABLE 2.—*Chemical composition of 4 kernel types at 12 stages of maturity in 1940* [1]

Carbohydrate	Kernel type	Days after pollination											
		12	14	16	18	20	23	26	29	32	38	50	59
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Reducing sugar	*Su Wx*	10.81	5.72	2.60	2.43	1.96	1.45	1.13	1.08	.98	.84	.92	.83
	su Wx	11.93	7.52	3.54	3.06	2.61	2.60	2.54	2.51	2.86	1.97	2.51	2.36
	Suwx	10.11	6.58	2.95	2.93	1.98	1.67	1.41	1.25	1.23	1.06	.91	.84
	suwx	12.97	8.28	4.48	3.78	3.13	2.44	2.45	2.74	2.92	2.27	2.68	2.49
Total sugar	*Su Wx*	23.04	13.61	6.87	6.76	6.28	5.45	3.88	3.52	3.31	2.20	2.68	1.52
	su Wx	23.19	18.05	12.56	11.45	10.12	9.38	7.79	6.60	6.59	2.78	5.33	4.63
	Suwx	26.45	15.99	7.74	7.80	7.44	5.34	5.03	4.58	4.51	2.82	2.78	1.98
	suwx	26.18	19.47	14.61	12.19	10.45	10.28	8.26	7.34	7.17	3.87	5.38	5.01
Water-soluble polysaccharides	*Su Wx*	2.87	2.26	2.27	3.28	4.34	5.12	4.05	4.28	4.34	4.81	5.11	6.60
	su Wx	5.16	13.47	18.93	32.20	33.48	33.77	36.49	37.10	37.41	31.25	33.04	27.95
	Suwx	3.07	2.97	3.37	3.76	6.90	8.15	5.42	6.40	7.18	7.64	9.79	8.19
	suwx	3.94	15.49	23.55	35.11	37.42	37.61	38.72	40.66	42.08	34.43	40.72	36.28

[1] Carbohydrates calculated as glucose and expressed as percentage of dry weight.

greater percentage of total sugars throughout. Waxy kernels showed a slightly higher percentage of total sugars than nonwaxy in 35 out of 40 comparisons within the sugary and starchy classes in both years. However, this consistent difference possibly may be due to removal of some dextrins by the 80 percent alcohol extraction; more being removed from the waxy than from the nonwaxy type.

When the residues which remained after removal of the sugars were shaken in water and allowed to stand 12 hours, they gave supernatant liquids which were very difficult to filter. The solution from the nonsugary kernels was almost transparent while that from the sugary kernels was cloudy. No differences in appearance of the supernatant

liquids were noted between waxy and nonwaxy types within the sugary and nonsugary classes.

Iodine tests were made on the water-soluble fraction for one harvest date in 1940, namely, at the 18-day stage. Four distinct tints resulted when a drop of very dilute IKI solution was used in making the test on a spot plate. If a drop of more concentrated IKI solution (0.3 percent potassium iodide and 1 percent iodine) is added four shades of red are obtained. A description of the color reactions is presented in table 3.

TABLE 3.—*Reaction of water-soluble fraction from 4 kernel types with iodine* potassium iodide

Kernel type	Color with dilute IKI	Color with concentrated IKI
Su Wx	Bluish purple	Brown-red.
su Wx	Grayish purple	Orange-red.
Suwx	Reddish	Purple-red.
uwx	Reddish yellow	Yellow-red.

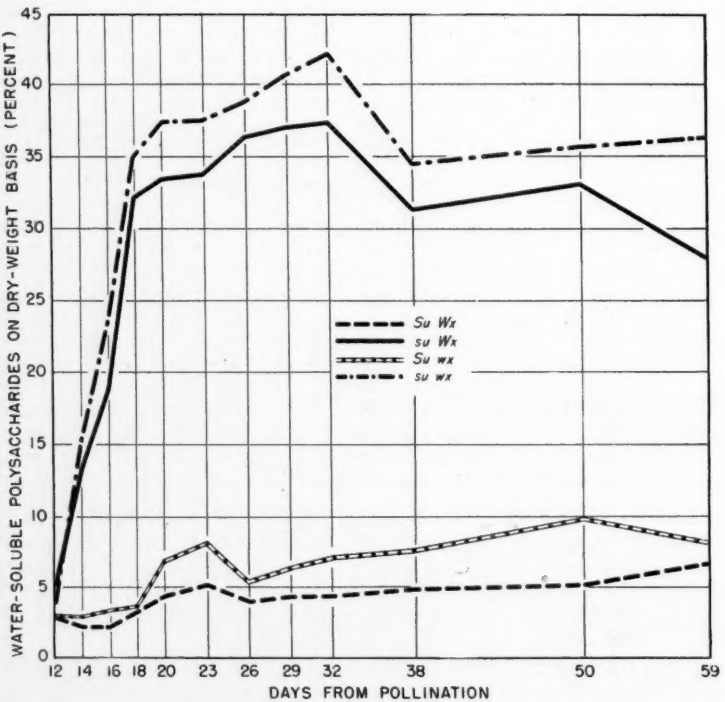

FIGURE 2.—Curves showing the changes in water-soluble polysaccharide content for four kernel types during development in 1940.

Purple tints obtained with dilute IKI for the nonwaxy endosperms indicate presence of some soluble starch in these varieties. The different shades of red with concentrated IKI indicate dextrinlike compounds in all four endosperms.

It is apparent from table 2 and figure 2 that the sugary gene is more influential than the waxy in determining the amount of the water-soluble fraction. In no case did the water-soluble fraction exceed 10 percent in the nonsugary kernels, whereas this percentage rose as high as 42.08 percent in the sugary types. The waxy gene, however, increases the water-soluble fraction in both the sugary and nonsugary classes.

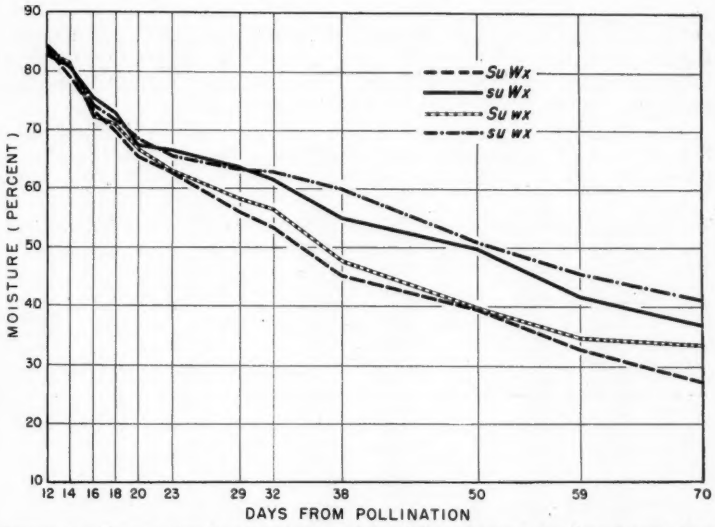

FIGURE 3.—Curves showing the changes in moisture content for four kernel types during development in 1940.

MOISTURE

Although the types of corn did not vary significantly in percentage of moisture at early stages of development, differences in the rates of moisture decline for the sugary and nonsugary classes became apparent after the 20-day stage (table 4 and fig. 3). The sweet types lose moisture less rapidly. Waxy kernels generally had a greater per-

TABLE 4.—*Moisture as percent in 4 kernel types at 15 stages of maturity in 1940* [1]

MOISTURE ANALYSIS

Kernel type	Days after pollination														
	5	8	10	12	14	16	18	20	23	29	32	38	50	59	70
Su Wx	90.52	88.34	87.33	83.66	79.07	73.50	69.60	65.80	62.32	55.93	53.35	45.11	39.44	32.78	26.67
su Wx	90.13	88.37	87.30	82.96	80.91	75.20	72.72	67.53	66.18	63.78	61.90	54.96	49.78	41.72	36.89
Su wx	90.64	88.89	87.63	83.10	81.31	72.66	71.18	66.41	63.09	58.26	56.61	47.87	39.93	34.77	33.86
su wx	90.36	88.28	87.57	84.10	80.94	73.97	71.60	68.12	66.07	63.73	62.97	60.00	50.52	45.80	41.06

[1] Minimum significant difference between means=2.21 percent.

TABLE 4.—*Moisture as percent in 4 kernel types at 15 stages of maturity in 1940*—Continued

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value
Harvests	14	6,604.0	2,096.51**
Kernel types	3	479.2	152.13**
Interaction (harvests × types)	42	37.6	11.93**
Error	240	3.2	
Total	299		

**=Significant at the 1-percent level.

centage of moisture than nonwaxy within the sugary and nonsugary classes, although all the differences are not statistically significant. The minimum significant difference in moisture content between any two types on any harvest date in 1940 is 2.21 percent.

It is of interest to note the length of time after pollination before the amount of dry matter in the different kernel types reaches 50 percent (table 5). The amount of dry matter reached 50 percent of

TABLE 5.—*Approximate time when dry matter reaches 50 percent in the 4 kernel types for the years 1940 and 1941*

Kernel type	Days from pollination In 1940		Days from pollination in 1941		Difference between years
	Non-sugary class	Sugary class	Non-sugary class	Sugary class	
					Days
SuWx	34		24		10
suWx		49		37	12
Suwx	36		29		7
suwx		51		41	10
Average	35	50	27	39	10

the total weight at earlier stages of development in the starchy kernels than in the sugary. In this respect starchy types averaged 15 days ahead of sugary types in 1940 and 12 days ahead in 1941. Within the nonsugary and sugary classes the nonwaxy types were ahead of the waxy types. It is to be noted that in 1941 the time required for the dry matter to reach 50 percent averaged 10 days less for all kernel types than in 1940. The difference may be attributed both to the weather conditions and to the fact that the ears in 1941 were not well filled.

KERNEL WEIGHT

That more reserves are stored in nonsugary than in sugary endosperms is shown by the comparative dry weights of the different kernel types at maturity. Seventy-two days after pollination ears were classified on a basis of endosperm type and number of kernel rows, making 12 classes in all. Absolute dry weights were obtained for 1,000 kernels of each class. Table 6 shows the average dry weight in grams for 100 kernels of each class and figure 4 illustrates the results. The nonsugary types attain greater dry weights than the

TABLE 6.—*Dry weight of 100 kernels of 4 endosperm types from 3 classes of ears 72 days after pollination*

Rows of kernels on ears (number)	Endosperm type			
	Su Wx	*su Wx*	*Suwx*	*suwx*
	Grams	*Grams*	*Grams*	*Grams*
14	30.93	22.85	30.34	23.64
12	31.89	24.62	31.27	25.02
10	34.07	26.26	33.82	26.11

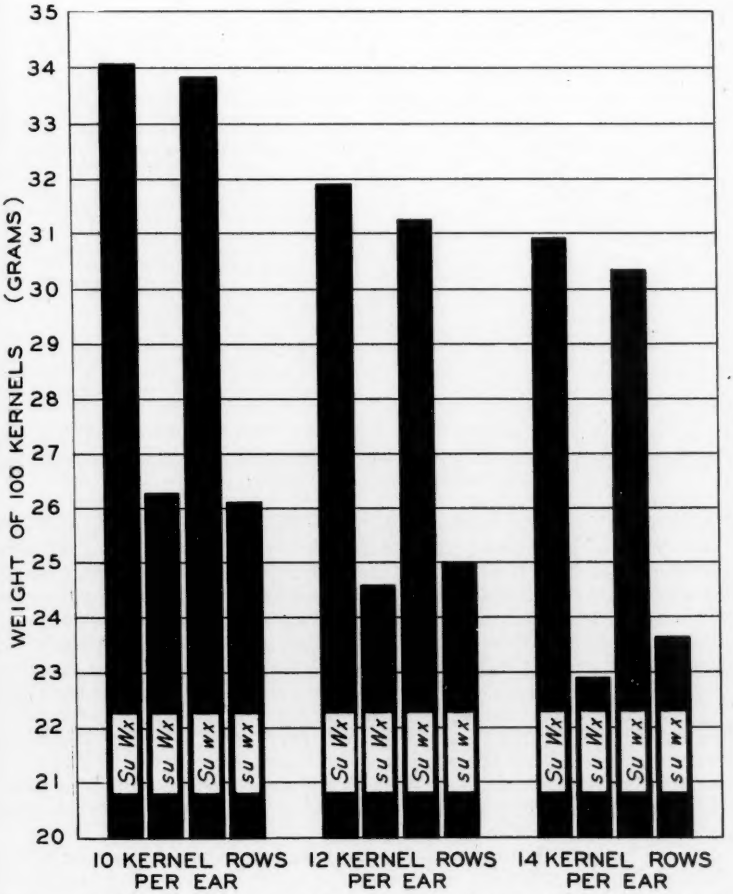

FIGURE 4.—Diagram showing graphically the dry weight of 100 seeds at the 72-day stage from ears with 10, 12, and 14 rows of kernels for each of 4 types of corn.

sugary types. For instance, *SuWx* kernels from 12-row ears had an average weight of 31.89 gm. per 100 seeds and *suWx* kernels, 24.62 gm. per 100.

TENDERNESS OF THE KERNEL

Data on resistance to puncture for the 4 kernel types which are presented in table 7 and in figure 5 represent the average values for

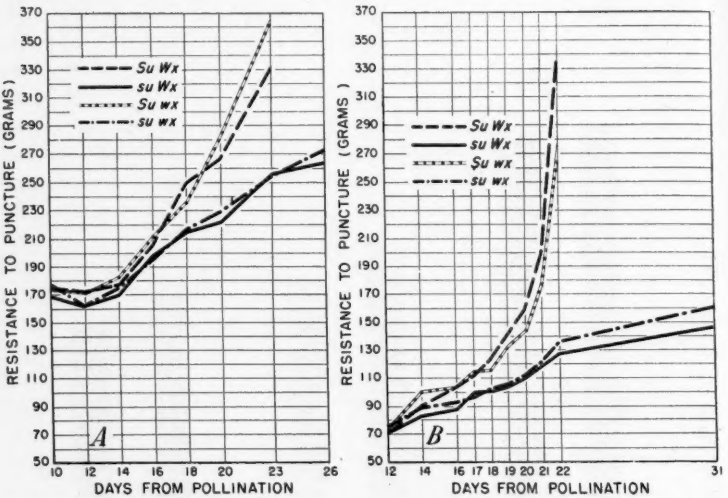

FIGURE 5.—Curves illustrating the increase in toughness of kernels for four types of corn in 1940 (*A*) and 1941 (*B*)

100 kernels (25 from each of 4 ears) in 1940, and for 120 kernels (15 from each of 8 ears) in 1941. Extensive discussion of the graphs is unnecessary, since it is obvious that the sugary and nonsugary endosperms fall into 2 distinct classes with respect to resistance to puncture. Nonsugary kernels were consistently tougher from the 12-day stage onward in 1940 and from the 14-day stage in 1941. At 26 days after pollination in 1940 and 31 days in 1941 it was impossible to measure a number of the nonsugary kernels because they offered more than 500-gm. resistance, the upper limit of the meter scale. There were no significant differences between the toughness of waxy and nonwaxy kernels within the sugary and nonsugary classes. Differences between any 2 kernel types for average resistance to puncture must be greater than 20 gm. in 1940, and in 1941, greater than 15 gm., in order to be statistically significant.

Since kernels with nonsugary endosperms proved to be much less tender than kernels with sugar endosperms, some related experiments were conducted in an attempt to evaluate the influence of the underlying tissue on the resistance which the pericarp offered to penetration. By 18 days after pollination the triploid endosperm tissue of maize occupies virtually all the space in the kernel except that taken by

the embryo. The ovary wall has been transformed into a comparatively thin pericarp (*19*).

TABLE 7.—*Average pressure in grams required for pericarp penetration of ¼ kernel types at 8 stages of maturity in 1940, and at 10 stages of maturity in 1941*

PRESSURE MEASUREMENTS, 1940 [1]

Kernel type	Days after pollination							
	10	12	14	16	18	20	23	26
SuWx	175	172	177	207	251	268	333	
suWx	169	162	170	198	216	223	256	264
Suwx	173	171	182	211	236	283	364	
suwx	177	162	175	197	217	229	255	273

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value
Harvests	6	39,512.0	192.53**
Kernel types	3	7,719.5	37.61**
Interaction (harvests × types)	18	1,579.9	7.70**
Error	84	205.2	
Total	111		

PRESSURE MEASUREMENTS, 1941 [2]

Kernel type	Days after pollination									
	12	14	16	17	18	19	20	21	22	31
SuWx	70	90	104	112	124	142	159	200	336	
suWx	71	83	88	99	101	105	111	120	129	148
Suwx	74	102	104	115	116	133	144	178	273	
suwx	76	89	93	97	103	106	112	122	136	161

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value
Harvests	8	59,487.1	262.95**
Kernel types	3	41,931.0	185.35**
Interaction (harvests × types)	24	8,193.5	36.22**
Error	252	226.2	
Total	287		

[1] Medium needle used (diameter = 0.048 inch). Minimum significant difference between means = 20 gm.
[2] Small needle used (diameter = 0.025 inch). Minimum significant difference between means = 15 gm.
**=Significant at the 1 percent level.

In one experiment two grades of paper, the so-called onionskin and 16-pound bond, respectively, were punctured over modeling clay of a heavy consistency and over window putty which was considerably more pliable. One hundred and fifty tests were made for each of the four combinations of paper and background. Table 8 shows the average values obtained in these tests. It is apparent that the underlying material, which corresponds to endosperm tissue in the corn kernel, has a profound effect on the puncture readings.

TABLE 8.—*Resistance to puncture offered by 2 types of paper over 2 types of background*

PUNCTURE MEASUREMENTS

Paper	Background	Average resistance
		Grams
Heavy	Modeling clay	444
Do	Putty	357
Onionskin	Modeling clay	223
Do	Putty	158

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value	Mean	Standard deviation	Coefficient of variation
				Grams		
Between treatments	3	1,711,982.75				
Within treatments:						
Heavy paper-clay background	99	967.54	1.92**	444	31.00	6.98
Heavy paper-putty background	99	1,062.96	2.11**	357	32.60	9.13
Onionskin paper-clay background	99	568.53	1.13	223	23.83	10.70
Onionskin paper-putty background	99	504.38	1.00	158	22.45	14.20
Total	399					

**=Significant at the 1-percent level.

A second experiment was designed to determine whether or not the sugary and starchy endosperm tissues had exerted a differential influence during development on the toughness of the hulls themselves. Pericarps from sugary, nonwaxy (*suWx*) and starchy, nonwaxy (*SuWx*) kernels at the 72-day stage were peeled and punctured. Peeling was accomplished by soaking the kernels for a short time in a dilute solution of sodium hydroxide, after which the tips were removed by a scalpel, and the hulls easily slipped off. Pieces of pericarp from the flat side of the kernel were laid over a washer held taut by a nut of small bore, and punctured. This simple test measured the resistance of the pericarp tissue alone, since there was no underlying support at the point of penetration.

Pericarps from sugary kernels offered an average resistance of 54 gm. for 250 tests, and those from nonsugary kernels, an average resistance of 75 gm. These differences are significant beyond the 1-percent point (table 9). Since the pericarps from both kernel types are alike

TABLE 9.—*Analysis of variance on resistance to puncture of peeled pericarps from sugary and nonsugary kernels*

Source of variation	Degrees of freedom	Variance or mean square	*F* value	Mean	Standard variation	Coefficient of variation
				Grams		
Between types	1	54,288.2	208.99**			
Within types:						
1. Su	249	265.6	1.09**	75	16.29	29.93
2. su	249	243.9		54	15.60	20.73
Total	499					

**=Significant at the 1-percent level.

genotypically, it must be assumed that they are influenced during development by the nature of the underlying endosperm tissue. The endosperm type is in turn determined by the type of pollen which is applied to the sugary, waxy parent (*suwx*). Swingle (*24*) has proposed the term "metaxenia" for the direct effect of the pollen on parts of the seed and fruit lying outside the embryo and endosperm. Waller (*25*), however, 11 years earlier, had proposed the term "ectogeny" for effects other than xenia, produced following fertilization. Such effects have been reported for the date palm (*18*), apple (*16 and 17*), cotton (*11*), and oak (*22*).

HISTOLOGICAL STUDIES

At the 16-day stage in 1940 the sugary and nonsugary kernels showed distinct differences in the nature and compactness of the storage products in the cells, but no differences were noted between waxy and nonwaxy kernels (fig. 6). The peripheral areas of all four endosperms, representing approximately one-third of the area in longi-

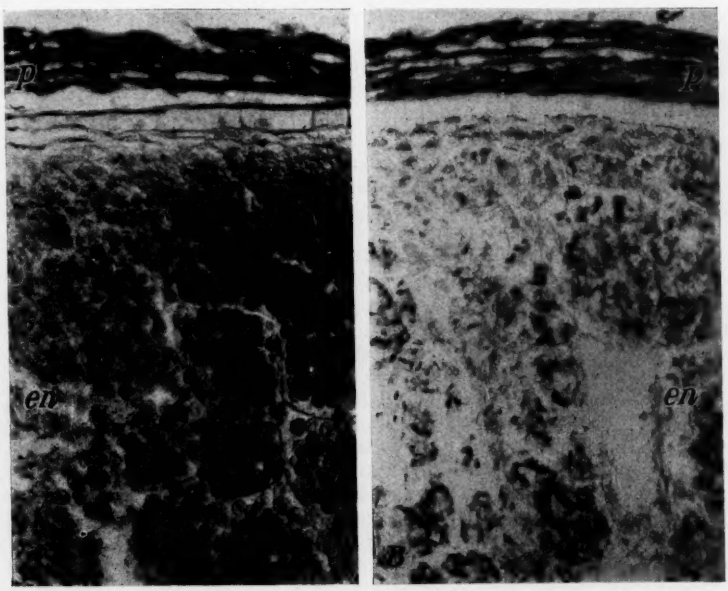

FIGURE 6.—Photomicrographs of longitudinal sections of corn kernels at the 16-day stage of development, showing the pericarp and the outer endosperm: *A*, *SuWx*; *B*, *suWx*; *p*, pericarp; *en*, endosperm. Ocular, × 10; objective, 16 mm.

tudinal section, were densely staining. In the nonsugary kernels, cells in this outer area were packed with simple starch grains (fig. 6, *A*). Toward the center of the kernel, however, there was a gradual decrease in the number of starch grains, until the cells in the basal region were without grains. On the other hand, the peripheral, storage region of the sugary kernels was composed of cells with two distinct types of reserves. Outer cells of this peripheral region were densely cytoplasmic, but showed no visible granules of solid carbohydrate

(fig. 6, *B*). Inner cells of the peripheral region were densely cytoplasmic, but they also contained compound granules of solid carbohydrate. Although these carbohydrate granules were compound, they were no larger than the simple starch grains of the nonsugary kernels; nor were they as numerous as the grains in the nonsugary types. Progressively toward the center of the sugary kernels, cells were lighter staining and contained a decreasing number of granules, until the basal region was clear and without grains.

CANNING TRIALS

Canning trials in 1940 were conducted at 3 stages of maturity, 14, 16, and 18 days after pollination. Between 50 and 100 ears of each type were canned as a whole-kernel pack. Brine consisting of 14 pounds of salt and 40 pounds of sugar per 100 gallons of water was used. The cans of corn were given code numbers and graded by 4 persons.

Fourteen days after pollination the corn proved to be decidedly too young for satisfactory commercial canning. While the moisture content of the four endosperm types was approximately the same, the nonsugary, nonwaxy kernels (*SuWx*) were slightly mealy, even at this early stage. This characteristic may be attributed to the endosperm reserves. Only 2.26 percent water-soluble polysaccharides were present and starch grains were already filling the cells. The three remaining kernel types (*suWx*, *Suwx*, and *suwx*) were closely similar in taste and texture. That the nonsugary, waxy type (*Suwx*) should be graded in a class with the sweet corns, is rather unexpected, for it too had a low percentage of water-soluble polysaccharides, and the endosperm cells were filled with starch grains.

At the 16-day stage the sweet types were still a little young for good quality canned corn. Nonwaxy, sweet corn (*suWx*) had the best flavor on this date, but in general it was very similar to the waxy, sweet type (*suwx*), although the latter was slightly more tender and watery. The nonsugary, nonwaxy type (*SuWx*) was lacking in flavor, but was still judged more like the sweet corns than like the nonsugary, nonwaxy type. The relative compactness of the storage products of sweet and starchy corn, shown in figure 6, makes the differences in texture readily understandable.

Eighteen days after pollination, two lots of each type were picked. One of these lots was allowed to stand outside the factory in bags for 24 hours and the other was canned immediately. Of the corn which was canned immediately, the sweet types proved to be at or near their optimum canning stage. The waxy, sweet variety (*suwx*) was in an optimum condition and was judged best in quality; it was also considered slightly better in quality than the nonwaxy, sweet type (*suWx*) which had been harvested at the 16-day stage, although the two were quite similar. The results of these trials show that the waxy gene retards the development of sweet corn slightly, but they do not indicate that it produces a longer optimum canning period, for the waxy sweet corn was a little young at the 16-day stage, and the nonwaxy sweet was a little old at the 18-day stage; nonwaxy sweet corn could have been ideally canned at 16 or 17 days after pollination, and the waxy sweet, at 17 or 18 days. At 18 days, the nonsugary, nonwaxy type was very mealy and decidedly undesirable

for eating purposes. The nonsugary, waxy type was strikingly different at this stage. It gave a thick, viscous product which resembled tapioca pudding in consistency and could not be poured from the can. It was relatively unpalatable.

After standing 24 hours, the two sweet corns gave canned products of a dull, flat flavor, indicating depletion of moisture and conversion of sugars and dextrins to starch. No differences in quality could be detected between the waxy and nonwaxy types.

DISCUSSION

Primarily the sugary and waxy genes influence the synthesis of polysaccharides in the endosperm cells of maize. Secondary effects are upon kernel weight, tenderness, and moisture content. Differences for all these qualities were greater between the sugary and nonsugary classes of kernels than between the waxy and nonwaxy classes.

Comment has already been made on the high percentage of water-soluble polysaccharides in sweet corn and the somewhat higher percentage of this fraction in the waxy, sweet kernels. It appears that introduction of the waxy gene into sweet corn varieties has promise as a means of increasing the water-soluble fraction which is so important in determining canning quality. On the basis of canning tests, waxy, sweet (*suwx*) was considered better than nonwaxy, sweet (*suWx*) when both types were harvested in optimum condition. On the other hand, while the delay in harvest, due to the waxy gene (*wx*), results in greater water-soluble polysaccharide content, it may also result in a tougher pericarp, since the waxy gene when homozygous delayed the optimum date of canning by a day.

It may be noted that a high percentage of sugar in the kernel is not of primary importance in determining canning quality. Sugars were present in maximum quantities for all types on the first dates of sampling, while the corn was not suitable for canning until several days later; differences between types were small.

The sugary gene when homozygous greatly reduces the resistance to puncture by modifying the nature and compactness of reserves in the endosperm cells. The differential influence of the *su* and *Su* genes on the resistance of the pericarp tissue itself, however, raises some interesting questions. Since the average dry weight attained by sugary kernels on 12-row ears was 24.62 gm. per 100, and by nonsugary kernels, 31.89 gm., it is apparent that absence of the nonsugary gene (*Su*) decreases the amount of total dry matter laid down in the endosperm. Do the effects of the *Su* and *su* genes in the endosperm also influence nutrition of the pericarp, since both the ovary wall and the endosperm are supplied by the same vascular tissue at the base of the kernel? Or does the difference in resistance have a mechanical explanation, the greater amount of reserves laid down in starchy endosperms resulting in greater stretching and increased growth of the pericarp?

The effects of the sugary and waxy genes on moisture depletion are interesting when it is recalled that the different kernel types developed on plants of the same genetic constitution. While it is undoubtedly true that maturity is controlled by many genes which influence such complex characters as growth rate, storage of food materials, and time of flowering, results of this work indicate that genes controlling endosperm type also influence the rate of maturation as measured by

moisture content. Further and more accurate studies could be made if mixtures of sugary and nonsugary pollen were applied to the same ears and moisture samples taken as soon as the kernel types could be classified.

SUMMARY

Two pairs of alleles, waxy (*wx*) and nonwaxy (*Wx*), and sugary (*su*) and nonsugary (*Su*), which influence storage products of the endosperm of maize are considered in this work. To obtain material for analysis under similar environmental conditions, four types of pollen, *SuWx*, *suWx*, *Suwx*, and *suwx* were applied under controlled conditions to *suwx* plants. Samples were taken periodically during development for studies on carbohydrate content, moisture content, dry weight, pericarp tenderness, morphology of the kernel, and canning quality.

Chemical studies bear out earlier work showing that sugary endosperms contain a greater amount of water-soluble polysaccharides than nonsugary endosperms. Presence of the waxy gene in sweet corn further increases the percentage of the water-soluble fraction.

Effects of the sugary and waxy genes are not confined to synthesis of carbohydrate reserves in the endosperm. The sugary kernels lose their moisture less rapidly with advancing maturity, and within the sugary and nonsugary classes, waxy kernels generally show greater percentages of moisture than nonwaxy kernels. At maturity, nonsugary kernels have a greater dry weight than sugary kernels.

Different kernel types fall into two distinct classes with respect to resistance to puncture, the resistance of the starchy kernels increasing much more rapidly with greater maturity than that of sugary kernels. This difference is largely due to the nature and compactness of reserves in the endosperm cells. However, the pericarps themselves, which are genotypically alike, give differences in resistance when they are peeled and punctured, those from nonsugary kernels offering more resistance than those from sugary kernels. No significant differences in toughness were noted between waxy and nonwaxy kernels within the sugary and nonsugary classes.

Canning trials made at three stages of development indicate that neither of the starchy endosperms (*SuWx* and *Suwx*) give desirable products. The waxy gene delays the optimum canning stage of sweet corn for a day without, however, increasing the duration of the canning stage.

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THE FERMENTATION OF CIGAR-LEAF TOBACCO AS INFLUENCED BY THE ADDITION OF YEAST

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INTRODUCTION

Tobacco of the cigar-leaf type must first undergo certain periods of fermentation before it can be used for cigar manufacture. The chemical changes that take place during fermentation involve, among others, a loss of some organic compounds and the synthesis of others with the final result that the aromatic and smoking qualities of the leaf are markedly improved.

Many theories have been advanced to explain the nature of the fermentation process. Among these may be mentioned the bacterial theory of Suchsland (*12*),[2] who claimed that certain micro-organisms have the power of acting upon leaf constituents and developing the characteristic flavor and aroma of fermented tobacco. The work of Behrens (*1*), Vernhout (*13*), and Koning (*4*) tends to support this theory.

Apparently, the first attempt to challenge the bacterial theory was made by Loew (*5*), who stated that leaf enzymes rather than bacteria provided the necessary mechanism of fermentation. For a long time this so-called enzyme theory of Loew's supplanted the bacterial theory. Recently, however, the work of Johnson (*2*) has revived the bacterial theory, and the later work of Reid and his coworkers (*6*, *7*, *8*, *9*, *10*) has proved conclusively that the major changes in fermentation are brought about through the activity of certain bacteria which usually develop in large numbers during the fermentation process, and that leaf enzymes play only a subordinate role.

The development of the micro-organisms of fermentation appears to be markedly affected by variations in the composition of the leaf. Some of these variations may occur during the curing process, but they may also be due in large measure to the environmental conditions under which the plants are grown, such as the nature of the soil, the season, and certain cultural practices which include the addition of fertilizers. In some cases the composition of the leaf may be of such a nature as to satisfy the nutrient requirements of undesirable rather than of desirable types of organisms. If, however, conditions are such as to favor the latter, the former do not appear

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[2] Italic numbers in parentheses refer to Literature Cited, p. 381.

to thrive. It seems obvious, therefore, that a stimulation of the desirable types of fermentation organisms should aid materially in obtaining a satisfactory fermentation, and for this reason an effort was made as herein reported to obtain such a condition by the addition of a material high in growth-accessory substances, namely, yeast, and to study the effect of such a treatment upon the microflora of the leaves during the preliminary stages of fermentation. No direct action of yeast on the fermentation process was expected as Street (*11*) had shown that yeast cells live but a short time after they are applied to leaf surfaces.

The use of yeast as a supplementary treatment of cigar-leaf tobacco prior to fermentation was first introduced by Koller (*3*) in 1858, and later was employed by other European workers. Recently, Street (*11*) reported on the use of yeast in the fermentation of domestic cigar-leaf tobacco. His results showed that the temperature rises in the earlier stages of fermentation were greater with all yeast treatments than when water alone was used and, furthermore, that yeast treatment produced a product which appeared to be more completely fermented in a given period of time as judged by appearance and smoking qualities than untreated samples. In his investigations, however, he gave no direct consideration to the effect of the treatment on the microflora of the leaves.

PLAN OF THE EXPERIMENTS AND MATERIALS USED

Preliminary experiments to determine the effect of yeast treatment on the fermentation of Pennsylvania cigar-leaf tobacco were first undertaken on representative samples of the 1940 crop. At the conclusion of these experiments similar studies were conducted on representative samples of the 1941 crop. For this work representative samples of freshly cured tobacco were obtained from Landisville and from the tobacco experimental laboratory at Roseville, Pa., on December 31, 1941.

The 1941 crop had not received sufficient rainfall for normal development and hence may be termed a "dry-weather" crop. Tobacco produced during a dry season is usually far more difficult to ferment than that produced under conditions of normal and well-distributed rainfall. Moreover, the 1941 tobacco did not cure well owing to the dry weather which prevailed after the crop was harvested.

In preparing samples for fermentation, five wooden cases, each 30 inches long, 9 inches wide, and 11.5 inches deep were used. Each case held 40 pounds of tobacco under 400-pound pressure. The yeast, when used, was applied to the samples in the form of a 10 percent aqueous suspension before the tobacco was placed in the case, and at the rate of 0.75 percent of the solid content of the weight of the tobacco. This quantity had been found in previous experiments to have no deleterious effect on the aroma of the tobacco. The yeast used represented a moist, freshly prepared, commercially cultivated strain of *Saccharomyces cerevisiae* of the type used in bread making.

EXPERIMENT 1

The treatments received by the tobacco both in the field and in the laboratory are presented in table 1. The tobacco in cases 4 and 5

came from five different experimental plots at the tobacco experimental laboratory, 8 pounds from each plot constituting the 40 pounds.

TABLE 1.—*Description of the tobacco samples used for fermentation studies (experiment 1)*

Sample No.	Case No.[1]	Where grown	Fertilizer treatment of crop		Supplementary laboratory treatment
			Pounds per acre	Formula	
1	1	Landisville	1,000	4-8-12	Control.
2	2	do	1,000	4-8-12	Yeast.
3	3	do	1,000	4-8-12	Do.
4	4	Roseville	1,000	3-9-24	Control.
5	5	do	1,000	3-9-24	Yeast.
6	4	do	1,000	3-9-12	Control.
7	5	do	1,000	3-9-12	Yeast.
8	4	do	1,000	3-6-12	Control.
9	5	do	1,000	3-6-12	Yeast.
10	4	do	1,000	3-9-18	Control.
11	5	do	1,000	3-9-18	Yeast.
12	4	do	1,000	3-12-12	Control.
13	5	do	1,000	3-12-12	Yeast.

[1] The tobacco used in cases 1 and 2 was from Kreider seed, that in case 3 from Kreider and Swarr seed mixed, and that in cases 4 and 5 from seed of a Swarr-Hibshman strain.

The tobacco was uniformly sprayed with water or yeast suspension and then exposed to the air for 24 hours. At the end of that period samples were taken for biological analysis. The results, representing the average of at least three samples in every case, are presented in table 2. In these and similar studies the bacteriological technique employed was that described by Reid, McKinstry, and Haley (*9*).

TABLE 2.—*Microflora of the tobacco before fermentation, moisture-free basis (experiment 1)*

Sample No.	Organisms per gram of tobacco			Nature and distribution of bacteria		
	Bacteria	Molds	Yeasts and yeastlike organisms	Cocci	Rods	
					Gram−	Gram+
	Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*
1	0.9	80	20	55	45	0
2	4.0	242	3,500	84	16	0
3	1.1	26	2,800	100	0	0
4	3.1	66	95	100	0	0
5	.2	34	1,300	0	50	50
6	.6	72	20	0	100	0
7	2.0	95	5,200	50	50	0
8	.1	50	30	0	100	0
9	7.0	57	13,200			
10	.3	32	40	0	50	50
11	4.0	67	16,300	0	33	67
12	.7	72	260	0	50	50
13	3.0	30	7,900	33	33	33

When the tobacco was placed in the fermentation chamber, a thermometer was inserted in the center of each case. The centers of cases 4 and 5 were occupied by samples 8 and 9, respectively. The changes in temperature noted are presented in table 3. An effort was made to obtain an environmental temperature of 72° F. at first, but certain mechanical difficulties were experienced before this temperature could be attained and stabilized.

A humidity of 75 percent was maintained for the first 27 days of fermentation. At the end of that period the temperature was increased approximately 20° F., and the humidity was raised to 80 percent. On the 71st day of fermentation, the temperature was lowered to approximately 80° F. and the humidity to 75 percent.

On the 93d day the tobacco was removed from the cases and aerated. Samples were taken at that time from each case for obtaining the judgment of a leaf expert in respect to its quality and the extent of this preliminary phase of fermentation. The remainder was remoistened and an effort was made to approximate 32 percent moisture in all samples. The tobacco was then placed in the original cases and returned to the fermentation chamber.

TABLE 3.—*Relation of yeast treatment to the temperature changes of fermenting cigar-leaf tobacco (experiment 1)*

Interval (days)	Room temperature	Degrees above room temperature in—				
		Case 1	Case 2	Case 3	Case 4	Case 5
	°F.	°F.	°F.	°F.	°F.	°F.
1	75	7	9	6	6	15
2	68	13	20	14	5	16
3	72	7	14	12	6	18
4	72	6	16	15	6	18
5	72	6	15	16	6	18
6	72	6	15	16	8	17
7	72	6	14	17	10	18
8	72	6	13	17	11	18
.	72	6	12	17	11	18
10	72	6	11	16	13	16
12	73	6	8	14	15	15
14	71	6	8	13	16	14
16	71	6	7	11	14	12
18	72	4	5	7	11	9
20	72	4	4	6	7	6
25	71	4	4	3	5	7
33	92	1	3	3	5	6
41	89	4	4	3	5	7
51	89	4	3	6	5	8
60	90	3	2	4	4	5
70	91	2	1	3	4	4
80	80	1	1	2	4	3
90	80	0	1	1	3	3
100	80	6	6	2	9	6
110	80	5	6	2	6	4
120	80	5	5	2	4	4

When the tobacco was removed from the cases and reconditioned following the 92d day of fermentation, an opportunity was afforded for studying the microflora of the individual samples listed in table 1. The results obtained are presented in table 6.

TABLE 4.—*Moisture content of the tobacco samples before and during fermentation (experiment 1)*

Case No.	Moisture content after fermentation—interval (days)					
	0	12	19	54	70	92
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	29.3	29.2	29.2	29.0	30.7	27.3
2	35.0	33.4	32.1	35.1	33.7	32.9
3	29.2	28.1	27.0	32.8	31.3	30.1
4	22.2	22.1	22.0	23.8	26.0	25.5
5	22.4	22.3	22.2	24.3	28.8	28.9

TABLE 5.—*Number and kind of organisms on samples of tobacco taken from cases at different intervals during the fermentation period (experiment 1)*

Interval (days)	Case No.	Organisms per gram of tobacco			Nature and distribution of bacteria			
		Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms	Rods	
							Gram−	Gram+
		Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*	*Percent*
12	1	180	300		100			
	2	2,390	500	1,000	100			
	3	1,250	760	1,100			100	
19	1	110	18		100			
	2	2,400	49	1,000	100			
	3	95	6,500	500	87		4	9
27	3	21	480	4,500	40	60		
	4	4	285	2,200	44	28		28
	5	5.2	800	4,000	100			
35	1	1,900	6	0	79	21		
	2	1,500	3	22	79	21		
	3	330	780	800	95	5		
	4	4.6	25	110	90	10		
	5	5.4	3,400	2,000	100	0		
54	1	1,230	4	0	100	0		
	2	410	.8	3	84	16		
	3	820	37	12	84	16		
	4	5	9	1,800	100	0		
	5	709	270	40	89	11		
70	1	995	7	60	91	9		
	2	560	(1)	30	100	0		
	3	1,100	6	3	95	5		
	4	1,300	30	67	91	9		
	5	2,110	21	680	95	5		

[1] Trace.

Samples of tobacco were taken by means of borings made into the center of the cases as the fermentation progressed. Moisture determinations were made on these samples before they were placed in the case, and on the 12th, 19th, 54th, 70th, and 92d day of fermentation. The results obtained are given in table 4.

Samples removed from cases at different intervals were analyzed to determine the number of organisms and the nature of those predominating. The results are given in table 5.

TABLE 6.—*Number and kind of organisms on the leaves of the 13 original samples of tobacco at the end of the 92d day of fermentation*

Sample No.	Case No.	Supplementary treatment	Organisms per gram of tobacco			Nature and distribution of bacteria	
			Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms
			Million	*Thousand*	*Thousand*	*Percent*	*Percent*
1	1	Control	590	<1	<1	87	13
2	2	Yeast	720	1	<1	63	37
3	3	do	740	1	<1	93	7
4	4	Control	220	1	310	93	7
5	5	Yeast	930	1	<1	87	13
6	4	Control	1,060	1	28	87	13
7	5	Yeast	420	1	<1	80	20
8	4	Control	680	1	75	63	37
9	5	Yeast	510	1	<1	87	13
10	4	Control	1,380	1	570	87	13
11	5	Yeast	650	1	96	93	7
12	4	Control	670	1	107	100	0
13	5	Yeast	1,960	1	<1	80	20

Tests conducted prior to fermentation indicated a good burn in the tobacco making up cases 1, 2, and 3; the burn of the tobacco in cases 4 and 5 was classed as fair. In every instance, however, the aroma was classed as poor.

The samples of tobacco taken at the end of the 92d day of fermentation and submitted to the judgment of an expert on cigar-leaf tobacco yielded the results shown in table 7.

TABLE 7.—*Burning quality, aroma, and fermentation of samples of tobacco on the 92d day of fermentation, as judged by an expert on cigar-leaf tobacco*

Case	Burn	Aroma	Degree of fermentation
1	Good	Poor	Not pronounced.
2	do	Fair	Fair to good.
3	do	Very mild	Very good; best of all samples tested.
4	Fair	Poor	Not pronounced.
5	Good	Good	Good; second only to sample 3.

EXPERIMENT 2

At the conclusion of the first experiment a second and somewhat similar experiment was instituted. For this experiment two cases were filled with cured tobacco from the southern part of Lancaster County and two with tobacco from the laboratory at Roseville. The Roseville tobacco came from duplicates of the plots from which the tobacco used in cases 4 and 5 of experiment 1 had been obtained. All these samples had been kept in bales for 4 months longer than those of experiment 1.

An effort was made to approximate a 28-percent moisture content and to maintain a humidity of 75 percent and a temperature in the fermentation chamber of 80° F. The experiment was started on May 2, 1942. As in the first experiment, samples were taken for analysis after the yeast and moisture treatment and before the tobacco was placed in the cases.

A description of the samples is given in table 8 and the results of the initial study of leaf organisms are presented in table 9. The temperature changes as influenced by yeast treatment are shown in table 10.

TABLE 8.—*Description of the tobacco samples used for fermentation studies (experiment 2)*

Sample No.	Case No.	Fertilizer treatment of crop		Supplementary laboratory treatment
		Pounds per acre	Formula	
20	11	1,000	4- 8-12	Control.
21	12	1,000	4- 8-12	Yeast.
22	13	1,000	3- 9-18	Control.
23	14	1,000	3- 9-18	Yeast.
24	13	1,000	3-12-12	Control.
25	14	1,000	3-12-12	Yeast.
26	13	1,000	3- 9-24	Control.
27	14	1,000	3- 9-24	Yeast.
28	13	1,000	3- 6-12	Control.
29	14	1,000	3- 6-12	Yeast.
30	13	1,000	3- 9-12	Control.
31	14	1,000	3- 9-12	Yeast.

TABLE 9.—*Microflora of the tobacco before fermentation, moisture-free basis (experiment 2)*

Sample No.	Case No.	Organisms per gram of tobacco			Nature and distribution of bacteria			
		Bacteria	Molds	Yeasts and yeastlike organisms	Cocci	Coccoid forms	Gram−	Gram+
		Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*	*Percent*
20	11	0.8	91	1	83	17		
21	12	2.2	950	520	91	9		
22	13	.8	9	53	100			
23	14	7.7	110	4,900	100			
24	13	.7	540	470	80		10	10
25	14	.7	131	1,400	100			
26	13	1.2	25	760	90			10
27	14	1.3	410	3,200	100			
28	13	.3	13	15	100			
29	14	9.2	58	8,400	100			
30	13	.1	30	36	100			
31	14	.7	90	4,100	100			

TABLE 10.—*Relation of yeast treatment to the temperature changes of fermenting cigar-leaf tobacco (experiment 2)*

Interval (days)	Room temperature	Degrees above room temperature in—			
		Case 11	Case 12	Case 13[1]	Case 14[1]
	°F.	°F.	°F.	°F.	°F.
1	81	3	11	4	4
2	81	5	12	4	4
3	80	7	15	6	11
4	80	8	16	6	13
5	80	9	17	6	14
6	80	9	17	6	14
7	80	9	16	7	13
8	80	9	15	7	11
9	80	9	12	6	10
10	80	8	10	5	7
12	81	7	9	5	6
14	80	7	9	6	6
16	80	7	8	5	5
18	80	5	6	4	5
20	80	5	6	4	5
25	80	5	6	4	5
30	80	5	7	4	5
40	80	5	7	5	5
50	81	3	4	2	2
60	80	3	3	3	1
70	80	2	2	2	0
80	80	2	1	1	0
90	80	2	1	1	0
100	80	1	0	1	0
110	80	0	0	0	0
120	80	0	0	0	0

[1] The center of cases 13 and 14 was occupied by samples 26 and27 respectively.

Samples were removed from the four cases on the 18th and 38th day of fermentation and were analyzed to determine the number of organisms and the nature of those predominating. The results are presented in table 11.

TABLE 9.—*Microflora of the tobacco before fermentation, moisture-free basis cases at different intervals during the fermentation period (experiment 2)*

Interval (days)	Case No.	Moisture	Organisms per gram of tobacco			Nature and distribution of bacteria	
			Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms
		Percent	*Million*	*Thousand*	*Thousand*	*Percent*	*Percent*
18	11	24.2	2.4	68	<1	93	7
	12	25.9	600	7	8,400	87	13
	13	24.7	400	20	4,000	100	0
	14	28.0	2,700	27	5,100	93	7
38	11	25.0	1,270	<1	210	100	0
	12	28.1	2,900	13	8	100	0
	13	23.7	1,390	4	109	100	0
	14	30.8	1,790	<1	3	73	27

Tests conducted before fermentation showed that the tobacco making up cases 11 and 12 possessed a good burn but poor aroma while the tobacco making up cases 13 and 14 possessed a fair burn and poor aroma. Examination of these particular samples, after 120 days of fermentation, indicated an improvement in both burn and aroma as a result of the yeast treatment, paralleling the results obtained in experiment 1.

DISCUSSION OF RESULTS

The addition of yeast to cigar-leaf tobacco under the conditions of the experiments herein described was reflected in a relatively high initial increase in temperature during the preliminary stages of fermentation, as indicated in tables 3 and 10.

An examination of the samples receiving yeast showed the presence of a number of true yeasts 24 hours after treatment, but no other occasion of sampling was the presence of these organisms noted. On all samples, however, the presence of certain yeastlike organisms was observed, whether or not the samples were treated with yeast.

The number of these organisms was definitely increased as a result of yeast treatment, which appeared to be beneficial to the fermentation process. Entirely different yeastlike organisms were first noted on samples taken from the cases used in experiment 1 at the end of the 92d day of fermentation. In this instance, however, the addition of yeast, on the whole, appeared to be antagonistic to the development of these particular organisms.

The experiments to determine the influence of yeast treatment on the bacterial flora of the tobacco samples yielded somewhat erratic results. Owing to the differences ordinarily prevailing in the chemical composition of the leaves and the difficulties encountered in obtaining uniform samples, this was to be expected. On the whole, however, the addition of yeast resulted in an increase in the number of bacteria during the preliminary stages of fermentation. With but few exceptions, a high bacterial count was paralleled by a low mold content, which is considered highly desirable. There appeared to be little, if any, increase in mold development as a result of yeast treatment.

SUMMARY AND CONCLUSIONS

The results of fermentation studies dealing with the application of bakers' yeast to Pennsylvania cigar-leaf tobacco of the 1940 and 1941 crop are presented. The following conclusions seem justified:

(1) Initial temperature gains were greater with all yeast-treated tobacco under the conditions of these experiments.

(2) The addition of yeast stimulated the development of organisms considered necessary to the fermentation process.

(3) A more rapid initial fermentation was evidenced in the case of the yeast-treated samples.

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VALUE OF SOYBEAN MEAL PREPARED FROM DAMAGED (BIN-BURNED) SOYBEANS AS A FEED FOR GROWING SWINE [1]

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INTRODUCTION

In Illinois during the spring and summer of 1942, many soybeans came out of storage in a badly damaged or "bin-burned" condition caused by an excess moisture. A sample of soybean meal was prepared by the expeller method from some of these damaged soybeans for comparison with normal expeller soybean meal prepared from mature soybeans that appeared sound after storage. The soybeans from which the bin-burned meal used in this test was prepared were described as "average 75 to 80 percent damaged." [3] At the Illinois station in the fall of 1942, a study was made of the feeding value of the bin-burned soybean meal in comparison with normal expeller soybean meal for growing-fattening pigs in dry lot.

METHODS

The method used consisted of feeding the pigs by the paired feeding technique for equal gains in order to obtain quantitative results. The writers believe this is the first time that paired feeding for equal gains has been attempted with swine. One pig of each pair received the check ration which contained normal soybean meal, while the other pig was fed the ration which contained bin-burned soybean meal.

The pigs were paired on the basis of weight, litter, sex, breed, type, condition, and probable outcome. Ten pairs of pigs were fed. They were purebred Chester Whites, Duroc Jerseys, Hampshires, and Poland Chinas raised on the University farm. Their weights varied from 28 to 80 pounds at the beginning of the test. The youngest pairs were 51 to 56 days old when the test began.

The basal ration used consisted of ground yellow corn, soybean meal, alfalfa meal, and mineral mixture. The mineral mixture was composed of 2 parts of ground limestone, 2 parts of steamed bonemeal, and 1 part of salt. The proportion of corn and soybean meal in the mixture was varied as the pigs increased in weight, whereas the alfalfa meal was fed at the 10-percent level and the mineral mixture was fed at the 2-percent level throughout the experiment. The changes made are indicated in table 1.

[1] Received for publication May 25, 1943.
[2] The assistance of R. H. McDade, chief swine herdsman, is gratefully acknowledged.
[3] "Bin-burned" soyean meal was prepared and donated to the University of Illinois by the Central Soya Company, Inc., Gibson City, Ill.

TABLE 1.—*Variations in the proportion of the ingredients of the rations fed the growing swine at different live weights*

Feeds	Proportion in which the feeds were mixed for pigs weighing—					
	75 pounds or less		75 to 125 pounds		Over 125 pounds	
	A [1]	B [2]	A [1]	B [2]	A [1]	B [2]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ground yellow corn	55	58	63	66	68	70
Normal soybean meal	33		25		20	
Bin-burned soybean meal		30		22		18
Alfalfa meal	10	10	10	10	10	10
Steamed bonemeal	.8	.8	.8	.8	.8	.8
Ground limestone	.8	.8	.8	.8	.8	.8
Sodium chloride	.4	.4	.4	.4	.4	.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1] Normal expeller soybean-meal mixture (check pigs).
[2] Bin-burned expeller soybean-meal mixture (test pigs).

The alfalfa meal was fed at the 10-percent level to insure against a possible vitamin deficiency in the all-vegetable diet. Recent experiments by the present writers [4] and by Ross, Phillips, Bohstedt, and Fargo at the Wisconsin station [5] indicate that higher levels of alfalfa meal than the usual 5 percent may be beneficial.

The chemical composition of the soybean meals used in the experiment, determined upon composite samples, is given in table 2.

TABLE 2.—*Chemical composition of the soybean meals on the fresh basis*

Feeds	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Normal soybean meal	88.10	42.50	4.27	5.04	4.86	31.43
Bin-burned soybean meal	93.41	46.00	4.60	5.60	6.34	30.87

In preparing the feed mixtures, the higher protein content of the bin-burned soybean meal was taken into consideration by equalizing the total percent of crude protein in the mixtures for the pigs in each weight group. As the pigs increased in weight, the ground corn and soybean meal were varied so that the following percentages of crude protein were fed: For pigs weighing 75 pounds or less, 20.3 percent; 75 to 125 pounds, 17.5 percent; over 125 pounds, 16.0 percent.

The pigs were fed twice daily in individual feeding crates. The feed mixture was weighed to one-tenth of a pound and fed in a metal trough with which each crate was equipped. A small amount of water was poured on the feed to prevent waste and to induce consumption. The pigs were watched closely in an attempt to keep the feed consumption up to the limit of the gains of the slowest-gaining pig of each pair. Records were kept of feed refusals. Individual weights of all pigs were taken at weekly intervals and the feed allowances for the following

[4] Unpublished data.
[5] WISCONSIN AGRICULTURAL EXPERIMENT STATION. USE 15% ALFALFA IN SOYBEAN OILMEAL PIG RATIONS. Wis. Agr. Expt. Sta. Bul. 453, pp. 18–20, illus. 1941.

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week were adjusted according to the gains made during the previous week.

The pigs were kept on concrete floors, the control pigs in one group and the pigs receiving the bin-burned soybean meal in another. The four small pigs of each group were also separated from the six larger pigs of the group. Each subgroup was given a pen in the central swine barn opening onto an outside concrete runway to which the pigs had access.

The experiment began on September 8, 1942, and was discontinued after 134 days, but before the pigs reached market weight, because the supply of bin-burned meal was exhausted.

RESULTS

The results of this experiment are summarized in table 3.

Table 3 shows that in 5 of the 10 pairs the test pig required more feed per pound of gain, while in 4 pairs the check pig required more feed. In 1 pair, the feed required for a unit of gain was the same. These results on economy of gain are too nearly a chance distribution to indicate any significant differences in the feeding value of the two rations. This was verified by statistical treatment of the data by Student's method with argument *t* as described by Snedecor.[6]

As would be expected, there was considerable variation in the economy of the gains among the various pairs. The largest differences in economy of gains existed between the pigs of pair 1 and pair 2. In these two pairs the check pigs made a unit of gain on 16.3 percent and 16.2 percent less feed respectively, than the test pigs.

In paired feeding for equal gains, it is not surprising that the average daily gains are less than the feed mixtures are capable of supporting under conditions of unrestricted feeding. Severe winter weather during the last 60 days of the test may have contributed to the slow gains. The check pig in pair 4, the pair with the lowest average daily gains, often had digestive disturbances, and also had more feed refusals than any other pig on test. This in part explains the small gains of the pigs in pair 4.

A record of feed refusals indicated that the feed mixtures were equally palatable.

The ideal in paired feeding for equal gains would be to have both pigs of a pair gain exactly the same weight every week. Since pigs vary greatly, in eating habits and other traits, even when strict paired methods are used, it is not surprising that weekly gains varied considerably. This variability may be noted in table 3. With 10 pairs of pigs fed for 19 weeks, a total of 190 weekly comparisons between the gains of pair mates is possible. Of the 190 weekly comparisons between gains of pair mates, 34 gave identical gains, while in 74 the check pigs gained more and in 82 the weekly gain favored the pigs fed bin-burned soybean meal. The deviation of 4 from the ideal of a chance distribution is considerably less than the standard deviation, and hence is not significant. When Student's method with argument *t* was calculated as described by Snedecor for weekly gains on each pair of pigs for the 19 weeks, the *t* values were very small and no statistically significant differences were noted. This indicates that the method of feeding for equal gains was successful.

[6] SNEDECOR, G. W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Rev. ed., 388 pp., illus. Ames, Iowa. 1938.

TABLE 3.—*Weights, gains, and feed consumption of 10 pairs of pigs, 1 of each pair being on the check ration (A) and the other on the bin-burned soybean-meal ration (B)*

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Pair 9		Pair 10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Final weight.....pounds..	156	147	118	125	99	105	95	94	158	163	181	181	178	168	114	124	179	184	161	164
Initial weight.....do....	49	42	28	34	32	33	42	40	52	55	67	69	70	59	42	49	76	80	72	70
Total gain.....do....	107	105	90	91	67	72	53	54	106	108	114	112	108	109	72	75	103	104	89	94
Period on test.....days..	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134
Average daily gain pounds..	.80	.79	.67	.68	.50	.54	.40	.40	.79	.81	.85	.84	.81	.81	.54	.56	.77	.78	.66	.70
Gain or loss in pounds after week No.:																				
1.....	1	−1	1	0	1	0	1	2	4	0	3	3	4	2	3	1	5	2	4	0
2.....	4	1	2	2	0	2	1	2	2	4	7	5	3	4	3	3	2	3	−1	2
3.....	4	3	4	2	4	1	1	1	3	2	1	−2	3	3	0	−2	4	5	3	3
4.....	4	3	0	1	3	2	1	1	6	2	5	6	6	8	2	5	6	6	7	7
5.....	6	5	4	5	3	5	1	4	5	8	7	7	8	6	4	7	8	7	3	5
6.....	3	6	6	2	4	3	3	0	2	3	5	5	5	5	5	3	0	3	5	0
7.....	3	5	2	5	2	4	2	4	4	5	5	4	5	7	6	6	6	7	6	7
8.....	7	3	6	5	7	3	1	2	6	5	4	4	9	10	5	5	8	8	5	4
9.....	5	6	6	5	2	6	2	1	5	5	5	7	3	3	8	6	4	3	3	6
10.....	2	5	7	6	4	6	1	4	7	7	9	7	14	9	4	9	8	9	8	1
11.....	2	4	1	4	0	−1	0	2	4	4	0	6	−1	5	−2	−3	1	6	4	7
12.....	8	10	7	8	4	7	7	3	7	8	10	6	7	8	5	4	7	4	1	1
13.....	10	5	7	4	6	5	6	2	8	6	4	7	7	5	3	4	7	4	0	6
14.....	8	8	9	7	7	5	2	5	8	8	10	13	10	13	9	9	12	10	8	8
15.....	10	6	6	6	6	5	5	4	7	8	11	6	9	8	7	9	1	8	8	9
16.....	0	1	6	6	5	7	4	3	1	2	0	2	−6	−5	−3	−6	9	1	11	11
17.....	9	12	12	13	3	3	10	9	8	8	5	7	7	2	7	10	7	7	8	1
18.....	6	8	2	1	3	3	2	1	8	10	10	7	5	7	3	3	1	3	4	6
19 [1].....	15	15	2	9	3	6	3	4	11	13	13	12	10	9	3	2	7	8	2	10
Total feed eaten...pounds..	351.5	412.5	283.9	342.1	259.8	279.7	248.5	227.7	416.3	464.2	443.9	487.5	513.9	497.7	347.0	345.0	502.7	488.2	388.8	429.8
Average ration.....do....	2.62	3.08	2.12	2.55	1.94	2.09	1.85	1.70	3.11	3.46	3.31	3.64	3.84	3.71	2.59	2.57	3.75	3.64	2.90	3.21
Feed consumed per pound of gain.....pounds..	3.29	3.93	3.15	3.76	3.88	3.88	4.69	4.22	3.93	4.30	3.89	4.35	4.76	4.57	4.82	4.60	4.88	4.69	4.37	4.57

[1] 8 days in this period. Initial and final weights are the averages of 3 weights taken on consecutive days. Single weights were used in the other cases.

SUMMARY

When a feed mixture of ground yellow corn, normal expeller soybean meal, alfalfa meal, steamed bonemeal, limestone, and sodium chloride was compared with a similar feed mixture in which expeller soybean meal prepared from bin-burned soybeans was fed to growing pigs in place of normal soybean meal, both mixtures were consumed readily. The paired feeding method for equal gains was used in this trial, which involved 10 pairs of young pigs. In 5 pairs of pigs, the check mate made more economical gains as measured by feed eaten per pound of gain. In 4 pairs, the mate fed bin-burned soybean meal gained more economically, while in 1 pair the economy of gains was the same. These results are too nearly a chance distribution to indicate any statistically significant differences in the feeding value of the two feed mixtures. From these data, it may therefore be concluded that the soybean meal prepared from the damaged soybeans was equal in feeding value to the soybean meal prepared from sound soybeans for growing-fattening pigs in dry lot.

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